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# Optimization and Evaluation of Biosurfactant Produced by *Pantoea sp*. Using Pineapple Peel Residue, Vegetable Fat and Corn Steep Liquor

Fabiola Carolina Gomes de Almeida[*1, 2], Thayse Alves de Lima e Silva[2], Ian Garrard[3], Leonie Asfora Sarubbo[4],Galba Maria de Campos-Takaki[2] and Elias Basile Tambourgi[1]

*1. School of Chemical Engineering, University of Campinas, Campinas 13083-852, Brazil*

*2. Nucleous of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, Recife 50.050-590, Brazil*

*3. Institute for Bioengineering, Brunel University, Uxbridge UB8 3PH, United Kingdom*

*4. Center of Sciences and Technology, Catholic University of Pernambuco, Recife 50.050-590, Brazil*

**Abstract:** In this study, the authors have investigated the potential of a bacterial strain of *Pantoea sp.*, isolated from wastewater of the textile industry, for the production of biosurfactant. The biosurfactant production was optimized by the combination of CCD (central composite design) and RSM (response surface methodology). To assess the effects and interactions of medium the vegetable fat (1.5, 2.0 and 2.5 v/v), the variables corn steep liquor (2.0, 5.0 and 8.0 v/v) and pineapple peel residue (10.0, 25.0 and 40.0 v/v) on the surface tension were evaluated. The empirical model developed through RSM in terms of the effective operational factors mentioned above was found to be adequate to describe the biosurfactant production. Compositional analysis of the produced biosurfactant has been carried out by FT-IR (Fourier transform infrared spectoscopy) and subjected to the test of removing hydrocarbons. Through the analysis, vegetable fat and pineapple peel residue were found to be the most significant factors, whereas corn steep liquor had less effect within the ranges investigated. A maximum reduction in surface tension of 30.00 mN/m was obtained under the optimal conditions of 2.0% (v/v) vegetable fat concentration, 5.0% (v/v) corn steep liquor and 25.0% (v/v) pineapple peel residue concentration of medium. FT-IR spectrometer analysis of the biosurfactant characterized it as a glycolipid derivative. The biosurfactant exhibited the ability to solubilize the hydrocarbons tested, working between 64% and 92%. According to consists of bars with a length proportional to the absolute value of the estimated effects divided by the standard error. On this chart, ANOVA (analysis of variance) effect estimates are arranged from the largest to smallest absolute value. The chart includes a vertical line at the critical p-value of 0.05. Effects for which the bars are smaller than the critical p-value are considered non-significant and do not have an effect on the response variables. The effects are either positive or negative ANOVA; the determination of regression coefficients and the construction of graphs were performed using the Statistical® program, version 7.0 (Statsoft Inc, RSA). The results, the biosurfactant produced by *Pantoea sp.* can be a valuable source for application in rapid environmental bioremediation.

**Key words:** Biosurfactant, optimization, bioremediation.

## 1. Introduction

Microbial surfactants or biosurfactants are surface active amphiphilic molecules produced by a number of microorganisms. They occur in nature as a diverse group of molecules comprising of glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, phospholipids, polymeric and particulate biosurfactants [1].

They are mainly produced by hydrocarbon-utilizing microorganisms exhibiting surface activity [2]. These molecules reduce surface tension and interfacial tension in both aqueous solutions and hydrocarbon mixtures. These properties create microemulsions, leading to micelle formation, in which hydrocarbons can be solubilized in water or hydrocarbon in water. They are popular in many fields: environmental, food

[*]**Corresponding author:** Fabiola Carolina Gomes de Almeida, doctorate student, research fields: biotechnology, microbiology, and bioremediation. E-mail: fabiolacgalmeida@hotmail.com.

industry and the biopharmaceutic technology areas, for example, for their interfacial, emulsifying, foaming, antimicrobial activity, their biodegradable nature, low toxicity and their temperature, pH and ionic strength tolerance [1, 3, 4]. But the high cost of production and recovery limit their use. Hence, to overcome this problem, scientists have focused on the development of novel production strategies, such as the formulation of novel production media, production on low-cost substrates and development of novel production approaches [5, 6].

Several studies report biosurfactant production on various agro-industrial solid byproducts under solid state fermentation [7, 8]. These permit an economic gain by reducing the production cost and by biotransformation of vegetative waste.

Petroleum hydrocarbons, one of the main sources of environmental pollution, pose a threat to marine life and to the terrestrial biota concentrated in free regions along the coastline [9]. The hydrocarbons are of environmental concern due to their toxic and carcinogenic effects in many animals, including human beings. In addition, they act as anti-estrogens in mammals and induce reproductive toxicity in women [10].

Bioremediation often appears as a viable tool in the environmental restoration of contaminated surfaces, especially when the contaminated area has great length and volume which inhibits using brushing and other technologies, and makes in situ remediation more attractive. The design of a bioremediation technology requires finding a biocatalyst (microorganism) possessing sufficient biodegradation activity and often also a method of increasing the bioavailability of hydrophobic pollutants for this selected microorganism. Culture conditions and the physiological state of cell populations can also significantly affect the ability to utilize contaminating pollutants [11, 12]. The use of biosurfactants in environmental applications has been quite promising because of their biodegradability, both in water and in the soil. They are also less toxic than chemical surfactants, clearly an advantage when cleaning contaminated soils. Effectively the application increases the biodegradation process, since the solubilization of low solubility compounds increase the bioavailability of these compounds to microorganisms [13]. Moreover, many of these processes, when combined with technologies such as soil washing, have low cost. Therefore, the objective of this study was to optimize the production of biosurfactant using *Pantoea sp*. grown by submerse fermentation in economic culture medium with a low cost nutrient, and to test its biosurfactant properties on contaminated soil.

## 2. Materials and Methods

### *2.1 Materials*

Vegetable fat was granted by a local snack bar in the city of Recife (state of Pernambuco, Brazil), stored according recommendations Chef and used without any further processing. Corn steep liquor was obtained from Corn Products do Brazil in the municipality of Cabo de Santo Agostinho, state of Pernambuco/Brazil. Diesel oil used in this study was obtained from local petrol pump (city of Recife, state of Pernambuco, Brazil) was filter sterilized and used in the studies. The sandy soil used in the process of bioremediation was obtained from the beach of Boa viagem, city Recife－Pernambuco/Brazil. The pineapple peel was collected in the commerce (Supply Central and Logistics of Pernambuco－CEASA).

### *2.2 Microorganisms*

The studies were conducted using *Pantoea sp.* isolated from wastewater of textile industrial laundry machinery located in Toritama-PE, Brazil, stored in the Culture Collection of the Center for Research in Environmental Sciences (NPCIAMB) Catholic University of Pernambuco, which is recorded in FCC (federation culture collection), It was stored at -22 ºC in brain-heart infusion broth medium supplemented

with 20% (v/v) glycerol solution.

*2.3 Culture Conditions and Medium Fermentation*

Pre-inoculation was carried out with *Pantoea sp.* grown in Petri dishes containing NA (nutrient agar), 5 g meat extract, 10 g peptone, 5 g NaCl, 17 g agar, distilled water 1,000 mL, pH 7.0, incubated at 30 °C for 24 h. Subsequently, they were removed from the lifted culture of *Pantoea sp.*, and transferred to Erlenmeyer flasks 125 mL capacity, containing 50 mL sterile distilled water until optical density at 600 nm corresponding 0.8 to an inoculum was obtained. The production of the biosurfactant was achieved using pineapple peel juice by grinding 300 g of pineapple bark in 400 mL of distilled water and then homogenising in a blender.

The homogenate was filtered in Whatman filter paper No. 1, and the pH was adjusted to 7.5 with a 0.1 M NaOH solution. Corn steep liquor, vegetable fat and juice from the pineapple peel residue were added according to the factorial design. Five percent aliquots (v/v) of the cell suspension were used to inoculate 500 mL erlenmeyer flasks containing 200 mL of sterile production medium. Cultivation was carried out at 30 ºC with agitation at 150 rpm for 72 h in a New Brunswick C-24 Shaker (New Brunswick Scientific, NJ, USA). At the end of the fermentation, samples were taken from the liquid culture to determine the surface tension. After selection of the best medium composition, the biosurfactant yield was determined, as described below.

*2.4 Optimization of Culture Conditions by RSM (Response Surface Methodology)*

Biosurfactant production was evaluated using an experimental design. A CCRD (central composite rotatable design) was used to determine the effects and interactions of the medium components with respect to the production of biosurfactant. Juice from the pineapple peel residue, corn steep liquor and vegetable fat concentrations were the independent variables.

Surface tension was the response variable. In this design, a set of 18 experiments was performed, with six replicates at the central points. The statistical analysis of the 4 replicates gives an indication of the experimental error of the production technique. The range and levels of the components (factors or independent variables) are given in Table 1. Each factor in the design was studied on five levels (−1.68, −1.0, 0, +1 and +1.68), with zero as the central coded value. These levels were based on results obtained in preliminary experiments. Based on the factorial design matrix, surface tension was studied with different combinations of the medium constituents.

The optimal values from the CCRD were obtained by solving the regression equation and analysing the response surface contour plots [3]. ANOVA (analysis of variance) with 95% confidence limits was used to determine the significance of the effects. The effects and significance of the variables were graphically studied using Pareto charts. A Pareto chart consists of bars with a length proportional to the absolute value of the estimated effects divided by the standard error. On this chart, ANOVA effect estimates are arranged from the largest to smallest absolute value. The chart includes a vertical line at the critical p-value of 0.05. Effects for which the bars are smaller than the critical p-value are considered non-significant and do not have an effect on the response variables. The effects are either positive or negative ANOVA; the determination of regression coefficients and the construction of graphs were performed using the Statistical® program, version 7.0 (Statsoft Inc, RSA).

*2.5 Determination of Surface Tension*

Changes in surface tension were monitored in the cell-free broth by initially centrifuging the cultures at 9,000 × g at 4 ºC for 15 min. Surface tension was then determined at room temperature using a Tensiometer from Sigma, KSV Instruments Ltd. model 70, Finland. Tension meters determine the surface tension using an

**Table 1 Experimental range and levels of the independent variable studied in the CCRD.**

| Test variables | Range and levels | | | | |
|---|---|---|---|---|---|
| | − 1.68 | − 1 | 0 | + 1 | + 1.68 |
| Vegetable fat (% v/v), $x_1$ | 1.16 | 1.5 | 2.0 | 2.5 | 2.84 |
| Corn steep liquor (% v/v), $x_2$ | 0.04 | 2.0 | 5.0 | 8.0 | 10.04 |
| Juice from the pineapple peel residue (% v/v), $x_3$ | 0.2 | 10.0 | 25.0 | 40.0 | 50.2 |

optimally wet table ring suspended from a precision scale. With the ring method, the liquid is raised until contact with the surface is registered. The sample is then lowered again so that the liquid film produced beneath the liquid is stretched.

Maximal force is determined as the film is stretched, this force is measured and used to calculate the surface tension. The instrument was calibrated against MilliQ-4 ultra pure distilled water (Millipore, Illinois, USA). Prior to use, the platinum plate and all glassware were sequentially washed with chromic acid, deionised water and acetone and flamed with a Bunsen burner.

### *2.6 Biosurfactant Isolation and Characterization by Spectrometer FT-IR (Fourier Transform Infrared Spectoscopy)*

The isolated biosurfactant was produced from the optimized conditions containing corn steep liquor, vegetable fat and juice from the pineapple peel residue. The cultures were centrifuged for 15 min at 10,000 × g to obtain cell-free supernatant, after subjected to the liquid-liquid extraction for isolation of procedure biosurfactant using different organic solvents such as chloroform/methanol (3:2:1), acetone (1:1), ethyl acetate (1:1) and ethanol (2:1) [14-16].

The foam formed on the surface part of each culture broth was collected and the residual solvent was removed by rotaevaporator. The lyophilized biosurfactant was weighed and the biosurfactant production was expressed in grams per liter of culture broth. FT-IR spectrometry was conducted on the isolated biosurfactant using 1.0 mg powdered with 1.0 g of KBr and then pressed with 7,500 kg for 30 s to produce a translucent KBr pellet. The FT-IR spectrum was recorded on a Nicolos Impact 410 system with a spectral resolution and wave number accuracy of 4 and 0.01 $cm^{-1}$, respectively. The IR spectra were collected from 500 to 4,000 wavenumber ($cm^{-1}$).

### *2.7 Application of the Biosurfactant in Hydrocarbon Removal from Contaminated Soil*

The sandy soil used in the study was collected from the semi-arid Geryon (Caatinga) located in Sierra Hewn, Pernambuco, Brazil. The physico-chemical analysis of the soil was performed in the soil agronomy department of the Federal Rural University of Pernambuco, Brazil. Glass columns (Spectra/Chrom, TX) with a diameter of 2.5 cm and a length of 10 cm were used. About 400 g sandy soil was impregnated with 200 mL of different oils, i.e. diesel oil, kerosene, petroleum and motor oil, the columns were packed with the contaminated soil containing the respective oils. The porous media was compacted using a stainless steel rod after every one-fifth of the column length was packed. The weight of the media packed in a column was measured to calculate the density and porosity. The bulk density and porosity of compacted soil were 1.428 ± 0.01 $g/cm^3$ and 0.356 ± 0.02 $g/cm^3$, respectively. The soil-packed column was then flushed with certain pore volumes of glycolipid solutions (1%, w/v) at a flow rate of 1 mL/min. During the flushing process, the effluent was collected using a fraction collector (Spectra/Chrom, TX) and the oil concentration was measured.

## 3. Results and Discussion

### *3.1 Optimization of Biosurfactant Production*

Medium composition such as vegetable fat, corn

steep liquor and pineapple peel residue are the factors that strongly influenced cell growth and the accumulation of biosurfactant, thus the optimization of these parameters can improve the bacterial efficiency. As mentioned, CCRD (central composite rotatable design) can be an excellent approach to study a process response and to figure out the best correlation among the parameters of a process. This is done via developed models based on the statistical methods to investigate the relationship between the inputs and outputs of any process. With the help of the CCRD, the authors execute the statistical models and evaluate the effect of parameters of a particular process as well as optimize the conditions for desirable responses. The CCRD is utilized as a statistical design to model the reduction in surface tension (biosurfactant production) in a process and to determine the significance of growth parameters and their interactions.

The factors affecting the biosurfactant production have been extensively studied in recent years, but few of these studies used statistical tools for experimental design. The classical method of medium optimization consists in changing one variable at time and keeping the others at fixed level. In this study the authors have optimized the growth conditions of the *Pantoea sp.* strain by using CCRD for designing the experiments, with aim of achieving highest possible rate of biosurfactant production. Due to the complex nature of biological processes, it is very difficult to predict exclusively the effects of all parameters, which may have multiple interactions. Therefore, CCRD was applied to build up an empirical model for biosurfactant production in terms of the operational parameters and concentration of vegetable fat, corn steep liquor and pineapple peel residue.

Design-Expert 7.1 suggested a quadratic Eq. (1) for the decrease in surface tension (Y):

$$Y = 30.4979 - 0.3468x_1 + 0.6207x_2 + 0.2479x_3 + 3.8427x_1^2 + 2.9060x_2^2 + 3.5776x_3^2 - 0.3500x_1x_2 - 0.1750x_1x_3 + 1.0250x_2x_3 \quad (1)$$

ANOVA results of the quadratic model in Table 2 revealed that the model equation derived by CCRD by Design-Expert 7.1 could adequately be used to describe the biosurfactant production under a wide range of operating conditions. According to the software and the specified section of optimization, the statistical confidence of the model is 95%, which is an appropriate value. The predicted versus experimental plot for surface tension showed that actual values were distributed close to the straight line (Fig. 1), ($R^2$ = 0.9086). Thus, it was a suitable model to predict the biosurfactant production efficiency using aforementioned experimental conditions.

The results in the present study revealed the optimal production of the biosurfactant by *Pantoea* sp. reached a maximum with concentrations of vegetable fat at 2.0% (v/v), corn steep liquor at 5.0% (v/v) and juice pineapple at 25% (v/v), the values observed under the experimental conditions are near to the values predicted by the model (Table 3). The biosurfactant of the *Pantoea sp*. under study was found with reduction

**Table 2 The analysis of variance(ANOVA) for the response surface quadratic model.**

| Source | Sum of squares | Degree of freedom | Mean square | F-value |
|---|---|---|---|---|
| Regression | 341.771 | 9 | 37.975 | |
| Waste | 72.173 | 8 | 9.022 | 4.209 |
| Lack of fit | 71.263 | 5 | 14.253 | |
| Pure error | 0.910 | 3 | 0.303 | 46.987 |
| Total | 413.940 | 17 | | |
| % explained variance = | | | 82.565 | |
| % maximum explainable variance = | | | 99.780 | |
| Correlation coefficient | | | 0.9086 | |

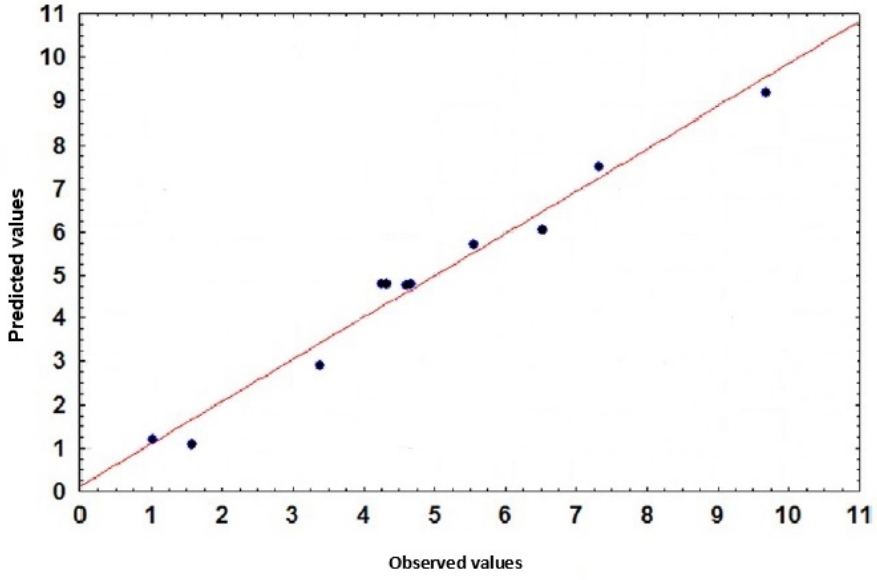


**Fig. 1 Observed values versus predicted values by model for the answer surface tension.**

**Table 3 Central composite design and corresponding responses.**

| Run | Fat vegetable (% v/v) | Corn steep liquor (% v/v) | Juice from the pineapple peel residue (% v/v) | Surface tension (mN/m) | |
|---|---|---|---|---|---|
| | | | | Experimental | Predicted |
| 1 | -1 | -1 | -1 | 38.20 | 42.04 |
| 2 | -1 | -1 | 1 | 41.36 | 40.84 |
| 3 | -1 | 1 | -1 | 39.61 | 39.45 |
| 4 | -1 | 1 | 1 | 42.54 | 42.35 |
| 5 | 1 | -1 | -1 | 41.89 | 42.40 |
| 6 | 1 | -1 | 1 | 39.98 | 40.49 |
| 7 | 1 | 1 | -1 | 38.51 | 38.41 |
| 8 | 1 | 1 | 1 | 41.00 | 40.60 |
| 9 | -1.682 | 0 | 0 | 42.02 | 41.95 |
| 10 | 1.682 | 0 | 0 | 40.00 | 40.79 |
| 11 | 0 | -1.682 | 0 | 41.01 | 39.76 |
| 12 | 0 | 1.682 | 0 | 38.00 | 37.68 |
| 13 | 0 | 0 | -1.682 | 40.90 | 40.20 |
| 14 | 0 | 0 | 1.682 | 40.80 | 41.04 |
| 15 | 0 | 0 | 0 | 30.80 | 30.50 |
| 16 | 0 | 0 | 0 | 30.60 | 30.50 |
| 17 | 0 | 0 | 0 | 31.00 | 30.50 |
| 18 | 0 | 0 | 0 | 30.00 | 30.50 |

of a surface tension of the medium of 69.67 mN/m for 30.00 mN/m one reduce of 56.93% (Table 3), which is comparable with the reports of Najafi et al. [17], when used which them bacterium are well known to produce biosurfactant, by thus it is capable of reducing surface tension of the media by approximately 30-60%.

Fig. 2 presents a graphic response surface of the effects of corn steep liquor, pineapple juice and vegetable fat on surface tension. As can be seen from Fig. 2 (a, b and c) the vegetable fat and pineapple juice had a definite influence on the production of biosurfactant. The production was drastically declined at higher

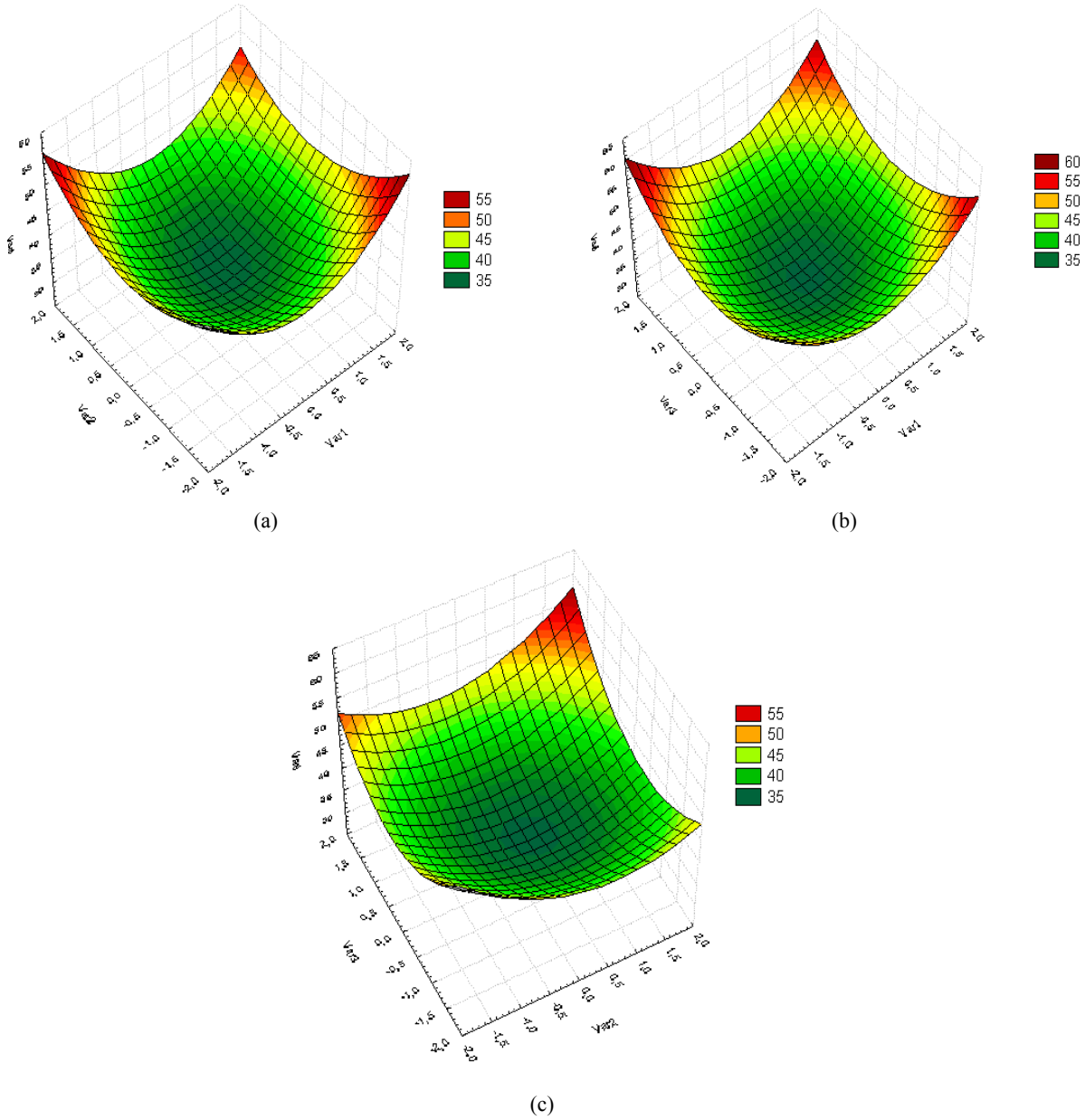


**Fig. 2 Response surface which shows the mutual influences of the levels of vegetable fat and corn steep liquor (a), vegetable fat and pineapple juice (b), and pineapple juice and corn steep liquor (c) on the surface tension of the medium.**

concentrations of vegetable fat and pineapple juice when compared to the lower concentrations, where the production was less affected. However, the corn steep liquor was influential in increasing production with the higher concentration value.

An ANOVA showed that the regression model had a high coefficient of determination ($R^2 = 0.9086$). This implies that 90% of the variation in the process efficiency is explained by the independent variables and also that only approximately 10% of the variation was not explained by the model. The model in Eq. (1) was then optimised. The optimal values of the process parameters were obtained in coded units, converted to uncoded units by using Eq. (1) and then experimentally validated, as shown in Table 4.

The results in the present study revealed the production of biosurfactant by *Pantoea sp*. in the range in which the bacterial strain was in stationary

**Table 4 Optimum values of the process parameters for the maximum process efficiency.**

| Parameter | Optimum value | |
|---|---|---|
| | Predicted | Experimental |
| Fat vegetable | 1.90 | 2.00 |
| Corn steep liquor | 5.30 | 5.00 |
| Pineapple peel residue | 20.05 | 25.00 |

phase, where the production of secondary metabolites. The availability of his substrates in optimal concentration provides the necessary amount of carbon, hydrogen and nitrogen which are elements needed for production of cells mass and biosurfactant for the biosynthesis and maintenance energy.

### *3.2 Biosurfactant Isolation and Characterization by FT-IR*

The FT-IR spectrum of the crude biosurfactant was investigated to gain insight into its chemical nature (Fig. 3). The results were compared with FT-IR spectral data of some known biosurfactants [6, 18-21]. The FT-IR analysis (Fig. 1) of the biosurfactants revealed presence of N-H at 2,302-2,348 $cm^{-1}$ respectively. Peaks observed at 1,659 $cm^{-1}$ correspond to the CO stretching vibrations of the carbonyl group, and C-O stretching. Peaks at 3,360-3,400 $cm^{-1}$ (of OH bonds) assigned to the carboxylic acid group of uronic acids. The presence of ester carbonyl bond was detected in the range at 1,300-1,000 $cm^{-1}$. The absorption of peaks at 846-848 $cm^{-1}$ can be due to the stretching vibration of the benzenoid ring. Presence of $CH_2$ group corresponds to peaks at 760-764 $cm^{-1}$ (Table 5). The FT-IR confirmed the glycolipid nature of the biosurfactants, which was similar to the earlier work by Worakitsiri et al. [18].

The carboxylic group is present as a functional group in the biosurfactant which confer its anionic character [22]. Furthermore, the carboxyl and sulfate groups provided overall negative charge to the biopolymer, thereby supporting binding and adsorptive properties for divalent cations by electrostatic interactions [23].

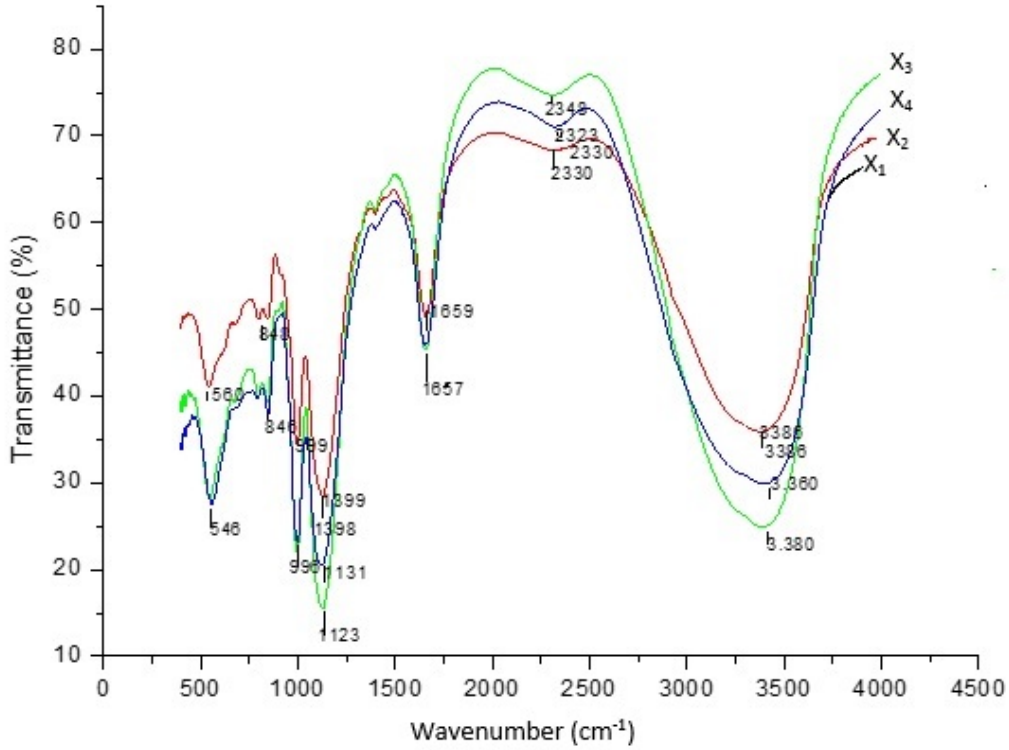


**Fig. 3 FT-IR spectra the biosurfactant extracted by different organic solvents; chloroform/methanol ($X_1$), acetone ($X_2$), ethyl acetate ($X_3$), ethanol ($X_4$).**

**Table 5 Characterization of the FT-IR spectra of the purified biosurfactant extracted from media.**

| Solvent extraction | Biosurfactant yield and chemical characterization | |
|---|---|---|
| | Biosurfactant (g/L) | FT-IR spectrometer |
| Chloroform/methanol | 3.43 | 3,380 $cm^{-1}$, 2,348 $cm^{-1}$, 1,659 $cm^{-1}$, 1,129 $cm^{-1}$, 996 $cm^{-1}$, 847 $cm^{-1}$, 796 $cm^{-1}$, 534 $cm^{-1}$ |
| Acetone | 3.35 | 3,360 $cm^{-1}$, 2,330 $cm^{-1}$,1,657 $cm^{-1}$, 1,131 $cm^{-1}$, 998 $cm^{-1}$, 846 $cm^{-1}$, 796 $cm^{-1}$, 546 $cm^{-1}$ |
| Ethyl acetate | 2.98 | 3,400 $cm^{-1}$, 2,323 $cm^{-1}$, 1,658$cm^{-1}$, 1,123 $cm^{-1}$,998 $cm^{-1}$, 848 $cm^{-1}$, 796 $cm^{-1}$, 546 $cm^{-1}$ |
| Ethanol | 2.65 | 3,386 $cm^{-1}$, 2,302 $cm^{-1}$, 1,659$cm^{-1}$, 1,398 $cm^{-1}$, 997 $cm^{-1}$, 847 $cm^{-1}$, 796 $cm^{-1}$, 546 $cm^{-1}$ |

**Table 6 Soil chemical characteristics.**

| Chemicals | Volumes |
|---|---|
| C | 19.2 g/Kg |
| N | 1.5 g/Kg |
| Calcium | 8.6 $cmol/dm^3$ |
| Magnesium | 3.55 $cmol/dm^3$ |
| Sodium | 0.01 $cmol/dm^3$ |
| Potassium | 0.15 $cmol/dm^3$ |
| Aluminum | - |
| Phosphorus | 2.0 $mg/dm^3$ |

**Table 7 Mass balance of oil at the end of the solubilization biossurfactant soil-packed column.**

| | Hydrocarbons | | | |
|---|---|---|---|---|
| | Petroleum | Kerosene | Diesel oil | Motor oil |
| Recovery (%) | 64.3% ± 0.1 | 89.6% ± 0.4 | 92.5% ± 0.5 | 77.6% ± 0.3 |

### *3.3 Application of the Glycolipid in Hydrocarbon Removal from Contaminated Soil*

The analysis of the physical characteristics of the sandy soil showed the presence of sand between 33 and 23 $g{\cdot}kg^{-1}$ among clay and 21 to 23 $g{\cdot}kg^{-1}$ according to the particle size test. For the gravimetric test 9.68% moisture was detected, and pH around 5.9. The study of the chemical composition of the soil was found to contain Calcium, Magnesium, Sodium, Potassium, Aluminum and Phosphorus according to Table 6. The total oil mass removed was calculated by converting the oil concentration into the mass and by extracting the residual oils in the soil after flushing to determine the overall removal efficiency. Fig. 1 shows the mass balance of the oils at the end of the solubilization experiment using 50 pore volumes of 150 mg/L glycolipid solution. The glycolipid solution removed between 64.3% and 92.5% of oils from the soil, respectively (Table 7). The study of Abouseoud et al., [24] showed that the solubility of biosurfactant parent in soil content naphthalene. Based on their results, the highest solubility was detected in the soil at pH 4.5-5.5.

Therefore, states that can biosurfactant produced present ability of different solubility hydrocarbons, for both high potential present in remediation of contaminated soil.

## 4. Conclusions

Sensitivity analysis was performed on the mathematical parameters to determine those that are most influential in the reduction of the surface tension and biomass as well as the increase in the yield of biosurfactant production. The most influential

parameters were optimised involving these studied conditions. Parameter estimation was performed to best fit the experimental data and the correlation coefficient obtained for surface tension. The present developments include the integrated optimization of the process, as well as the extension of the model to address the cultural conditions for biosurfactant production from *Pantoea sp.* the biosurfactant produced appears promising for applications in bioremediation processes.

The ability in recovering the oil from oil-saturated sand was also demonstrated. These characteristics indicate the potential use of the biosurfactant in the oil industry, especially in MEOR (microbial enhanced oil recovery). Studies are in progress to scale up the growth conditions and biosurfactant production in the bioreactors.

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## References

[1] Xu, Q., Nakajima, M., Liu, Z., and Shiina, T. 2011. "Biosurfactants for Microbubble Prepartion and Application." *Int. J. Mol. Sci.*12: 462-75.

[2] Chen, C. Y., Chiang, J. T., Liang, W. T., Wang, L. I., and Wang, L. S. 2012. "Reclamation of Squidpen by *Bacillus licheniformis* TKU004 for the Production of Thermally Stable and Antimicrobial Biosurfactant." *Biocatal. Agric. Biotechnol*. 1: 62-9.

[3] Pacwa-Płociniczak, M., Grażyna, A., Płaza, G. A., Seget-Piotrowska, Z., and Cameotra, S. S. 2011. "Environmental Applications of Biosurfactants: Recent Advances." *Int. J. Mol. Sci.* 12: 633-54.

[4] Satpute, K. S., Banat, M. I., Dhakephalkar, K. P., Banpurkar, G. A., and Chopade, A. B. 2010. "Biosurfactants, Bioemulsifiers and Exopolysaccharides from Marine Microorganisms." *Biotechnol. Adv*. 28: 436-50.

[5] Ismail, W., Al-Rowaihi, S. I., Al-Humam, A. A., Hamza, Y. R., El Nayal, M. A., and Bououdina, M. 2012. "Characterization of a Lipopeptide Biosurfactant Produced by a Crude-oil-emulsifying *Bacillus sp*. I-15." *Int. Biodeterior. Biodegrad.* 30: 1-11.

[6] Oliveira, F. W. D., França, L. W. I., Félix, N. K. A., Martins, L. J. J., Giro, A. E. M., and Melo, M. M. V., et al. 2013. "Kinetic Study of Biosurfactant Production by *Bacillus Subtilis* LAMI005 Grown in Clarified Cashew Apple Juice." *Colloids Surf. B: Biointerf*. 101: 34-43.

[7] Amani, H., Sarrafzadeh, M. H., Haghighi, M., and Mehrnia, M. R. 2010. "Comparative Study of Biosurfactant Producing Bacteria in MEOR Applications." *J. Pet. Sci. Eng.* 75: 209-14.

[8] Gesheva, V., Stackebrandt, E., and Tonkova, V. 2010. "Biosurfactant Production by Halotolerant Rhodococcus Fascians from Casey Station, Wilkes Land, Antarctica." *Curr. Microbiol*. 61: 112-17.

[9] Martino, C. D., López, N. I., and Iustman, L. J. R. 2012. "Isolation and Characterization of Benzene, Toluene and Xylene Degrading *Pseudomonas sp*. Selected as Candidates for Bioremediation." *Int. Biodeterior. Biodegrad*. 67: 15-20.

[10] Gudiña, E. J., Pereira, J. F. B., Rodrigues, L. R., Coutinho J. A. P., and Teixeira J. A. 2012. "Isolation and Study of Microorganisms from Oil Samples for Application in Microbial Enhanced Oil Recovery." *Int. Biodeterior. Biodegrad*. 68: 56-64.

[11] Cooper, D. G. and Goldenberg, B. G. 1987. "Surface Active Agents from Two *Bacillus* Species." *Appl. Environ. Microbiol*. 53: 224-9.

[12] Yan, P., Lu, M., Yang, Q., Zhang, H. L., Zhang, Z. Z., and Chen, R. 2012. "Oil Recovery from Refinery Oily Sludge Using a Rhamnolipid Biosurfactant-producing Pseudomonas." *Bioresour. Technol.* 116: 24-8.

[13] Chrzanowsky, Ł., Dziadas, M., Ławniczak, Ł., Cyplik, P., Białas, W., and et al. Szulc, A. 2012. "Biodegradation of rhamnolipids in liquid cultures: Effect of Biosurfactant Dissipation on Diesel Fuel/B20 Blend Biodegradation Efficiency and Bacterial Community Composition." *Bioresour. Technol.* 111: 328-35.

[14] Ilori, M. O., Amobi, C. J., and Odocha, A. C. 2005. "Factors Affecting Biosurfactant Production by Oil Degrading Aeromonas Spp. Isolated from a Tropical Environment." *Chemosph.* 61: 985-92.

[15] Paraszkiewicz, K., Kanwal, A., and Długon, S.´J. 2002.

"Emulsifier Production by Steroid Transforming Flamentous Fungus *Curvularia Lunata* Growth and Product Characterization." *J. Biotechnol.* 92: 287-94.

[16] Takahashi, M., Morita, T., Wada, K., Hirose, N., Fukuoka, T., and Imura, T. 2011. "Production of Sophorolipid Glycolipid Biosurfactants from Sugarcane Molasses Using *Starmerella Bombicola* NBRC 10243." *J. Oil Sci.* 5: 267-73.

[17] Najafi, A. R., Rahimpour, M. R., Jahanmiri, A. H., Jahanmiri, R., Roostaazad, D., and Arabian, Z. G. 2010. "Enhancing Biosurfactant Production from an Indigenous Strain of Bacillus Mycoides by Optimizing the Growth Conditions Using a Response Surfasse Methodology." *Chem. Eng. J*. 163: 188-94.

[18] Worakitsiri, P., Porsunthorntawel, O., Thanpitcha, T., Chavadej, S., Weder, C., and Rujiravanit, R. 2011. "Synthesis of Polyaniline Nanofibers and Nanotubes of Polyniline Nanofibers and Nanotubes via Rhamnolipid Biosurfactant Templating." *Synth. Met*. 161: 298-306.

[19] Wei, H. Y., Wang, L. C., Chen, W. C., and Chen, S. Y. 2013. "Production and Characterization of Fengycin by Indigenous Bacillus Subtilis F29-3 Originating from a Potato Farm." *Int. J. Mol Sci*. 11: 4526-38.

[20] Ragheb, A. A., Sandrin, T. R., Bodour, A. A., Maier, R. M. 2000. "Rhamnolipid-Induced Removal of Lipopolysaccharide from Pseudomonas Aeruginosa: Effect on Cell Surface Properties and Interaction with Hydrophobic Substrates." *Appl. Environ. Microbiol*. 5: 3262-8.

[21] Faria, A. T., Martinez, D. S. T., Barbosa, O. N. G., Vaz, G. B., Silva, S. I., and Garcia, S. J. et al. 2011. "Production and Structural Characterization of Surfacten ($C_{14}$/$Leu_7$) Produced by *Bacillus Subtilis* Isolate LSFM-05 Grown on Raw Glycerol from the Biodiesel Industry." *Process Biochem.* 46: 1951-7.

[22] Kitamoto, D. 2008. "Naturally Engineered Glycolipid Biosurfactants Leading to Distinctive Self-assembling Properties." *Yakug Zasshi.* 5: 695-706.

[23] Tonkova-vasileva, E., and Gesheva, V. 2007. "Biosurfactant Production by Antarctic Facultative Anaerobe *Pantoea ps*. during Growth on Hydrocarbons." *Curr. Microbiol*. 54: 136-41.

[24] Abouseoud, M., Yataghene, A., Amrane, A., and Maachi, R. 2010. "Effect of pH and Salinity on the Emulsifying Capacity and Naphthalene Solubility of a Biosurfactant Produced *by Pseudomonas Fluorescens*." *J. Hazard. Mater*. 80: 131-6.